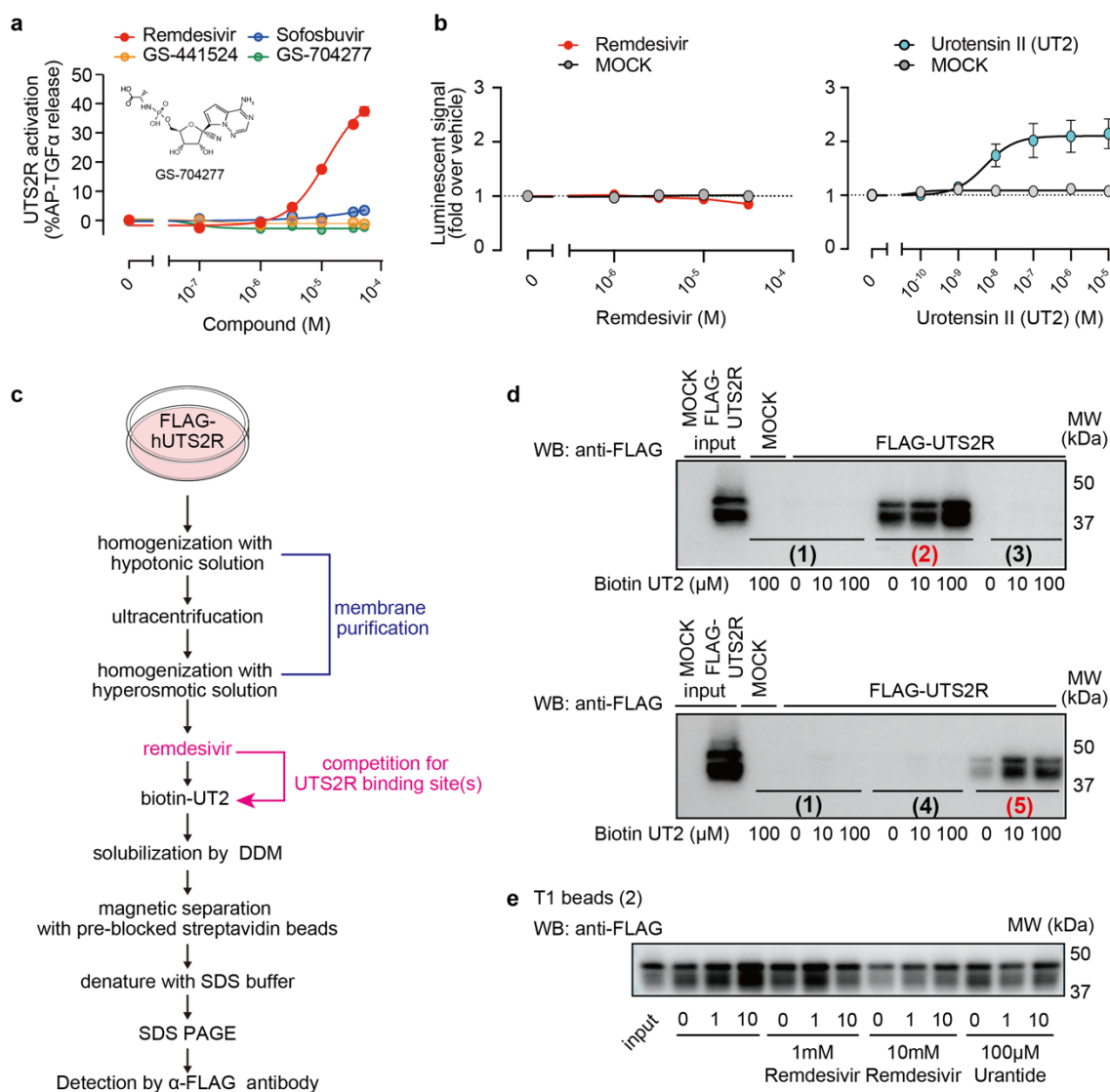


Supplementary Information

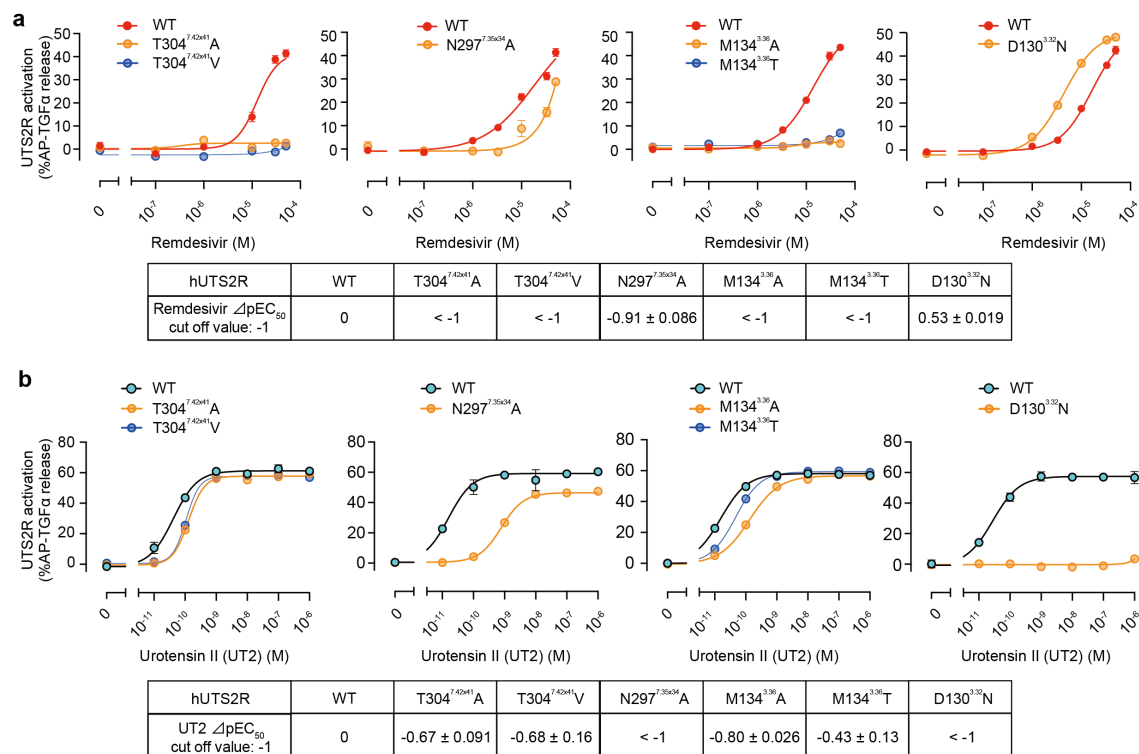
Supplementary Figure 1. Activation of urotensin-II receptor (UTS2R) by remdesivir.



(a) Remdesivir-related compounds-mediated TGFα-shedding response curves for UTS2R. Data are shown as means ± SEM (n = 3). (b) (left) Remdesivir- or (right) urotensin-II (UT2)-mediated β-arrestin 2 recruitment assay for UTS2R. Data are shown

as means \pm SEM (n = 3). **(c)** Experimental procedures of competitive binding assay. **(d)** Comparison of streptavidin magnetic beads. (1) Streptavidin magnetic beads (NEB, hydrophilic), (2) Dynabeads MyOne Streptavidin T1 (hydrophobic), (3) Dynabeads MyOne Streptavidin C1 (hydrophilic), (4) Dynabeads M-270 Streptavidin (hydrophilic), (5) Dynabeads M-280, Streptavidin (hydrophobic). Hydrophobic beads (2) or (5) served the aim for competitive binding assay. **(e)** UTS2R-bound biotin-UT2 detection using T1 beads (2). Membrane fraction of HEK293 cells expressing FLAG-UTS2R (input) was incubated with biotin-UT2 in the presence or absence of remdesivir or urantide. FLAG-UTS2R was then pulled down by streptavidin-coated T1 magnetic beads, and the amount of FLAG-UTS2R was detected by western blot.

Supplementary Figure 2. Effects of mutations on UTS2R activation.

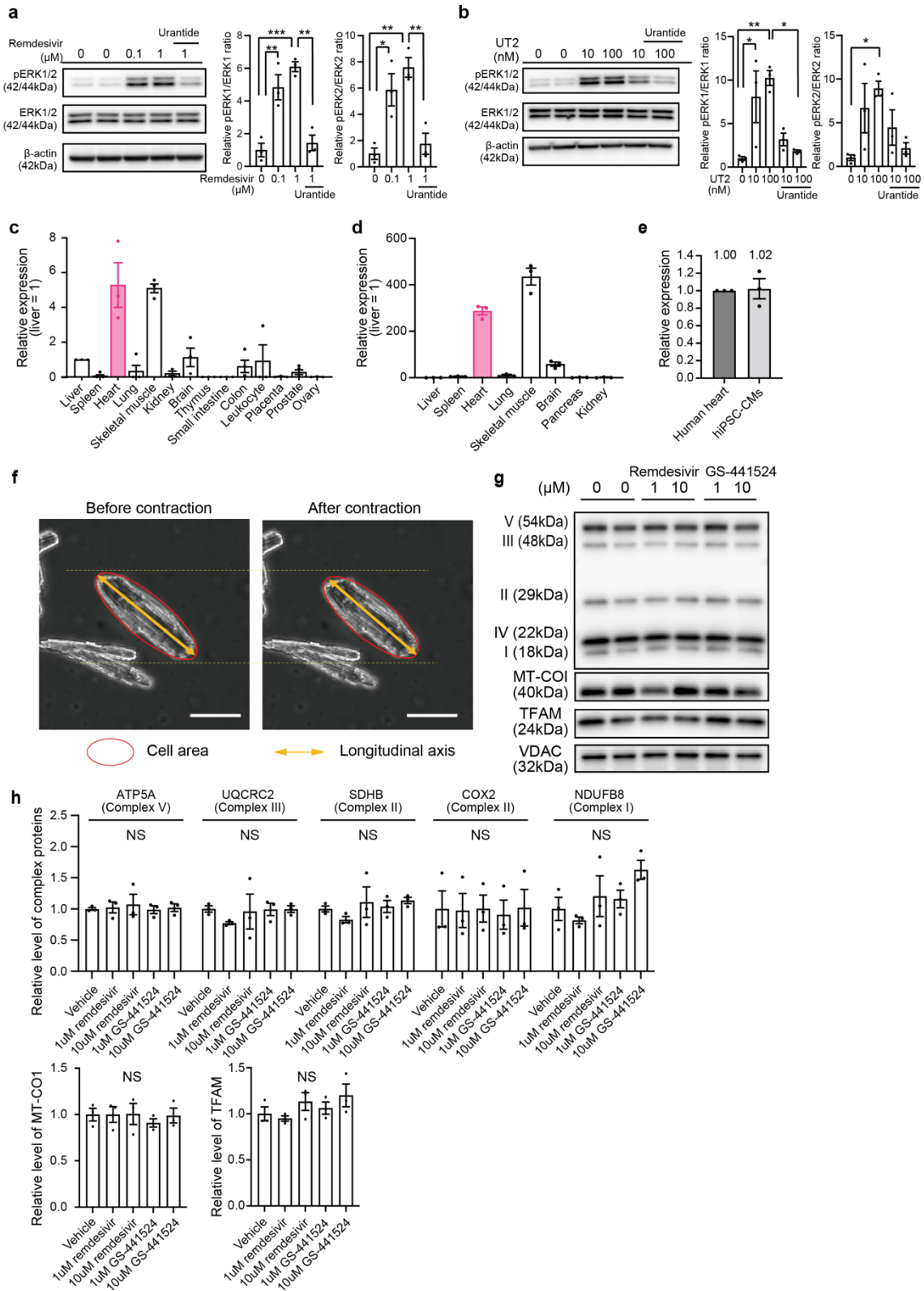


(a, b) Remdesivir (a)- and UT2 (b)-mediated TGF α -shedding response curves for UTS2R

WT and indicated mutants. ΔpEC_{50} value = pEC_{50} mutant - pEC_{50} WT. The ΔpEC_{50} cutoff value was set to -1. EC_{50} values were determined by the TGF α -shedding assay. Data are shown as means \pm SEM (n = 3).

Supplementary Figure 3. Intracellular signaling evoked by remdesivir-mediated

UTS2R activation.

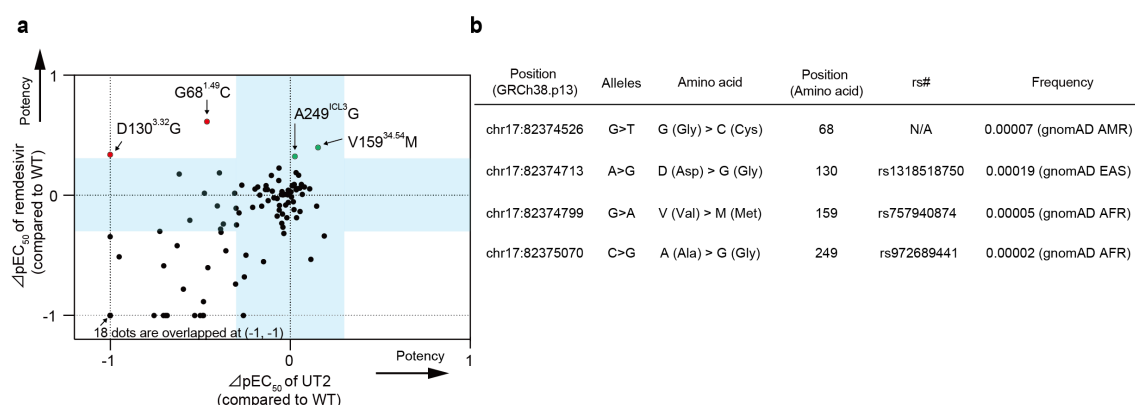


(a) Serum-starved HEK293 cells overexpressing UTS2R were stimulated with the indicated concentrations of remdesivir for 72 h with or without urantide, a UTS2R antagonist, and the lysates subjected to western blotting analysis. ERK1 and ERK2 activation ratio (pERK1/ERK1 and pERK2/ERK2) were calculated with data normalized to the vehicle. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Tukey's multiple comparisons test. Data are shown as means \pm SEM ($n = 3$). **(b)** Serum-starved HEK293 cells overexpressing UTS2R were stimulated with the indicated concentrations of UT2 for 5 min with or without urantide, a UTS2R antagonist, and the lysates subjected to western blotting analysis. ERK1 and ERK2 activation ratio were calculated with data normalized to the vehicle. * $p < 0.05$, ** $p < 0.01$ by Tukey's multiple comparisons test. Data are shown as means \pm SEM ($n = 3$). **(c)** Relative expression levels of UTS2R in normal human tissues were assessed by qPCR using the Human Multiple Tissue cDNA (MTC) panel. Data are shown as means \pm SEM ($n = 3$). **(d)** Relative expression levels of Uts2r in adult mouse tissues as assessed by qPCR. Data are shown as means \pm SEM ($n = 3$). **(e)** Comparison of relative gene expression levels of UTS2R between human heart (using MTC panel) and human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Data are shown as means \pm SEM ($n = 3$). **(f)** Evaluation of adult cardiomyocyte contractile activity. Percentile change in cell area was calculated by dividing the cell area before contraction

by that after contraction. Ratio of cardiomyocyte shortening was calculated by dividing the longitudinal axis before contraction by that after contraction. Scale bars = 50 μ m. **(g,**

h) Expression levels of mitochondrial respiratory complex proteins in cells treated with remdesivir or GS-441524. HEK293 cells overexpressing UTS2R were stimulated with the indicated concentrations of remdesivir or GS-441524 for 48 h. For each cell lysate sample, 10 μ g of proteins were subjected to SDS-PAGE and western blotting. Each cropped image was taken from a separate blot. Mitochondrial respiratory complex proteins, namely MTCO1, TFAM, and VDAC were detected on separate membranes, respectively. The intensity of respiratory complex proteins, MTCO1, and TFAM were normalized to VDAC (single VDAC control with the same protein loaded (10 μ g per lane, not run on each blot) and calculated with data normalized to the vehicle by Tukey's multiple comparisons test. Data are shown as means \pm SEM (n = 3).

Supplementary Figure 4. Effects of SNV on remdesivir-mediated UTS2R activation.



(a) Scatter plot of ΔpEC_{50} of remdesivir vs. ΔpEC_{50} of UT2 for 110 missense human SNVs. Light blue bands represent the range of $-0.3 < \Delta pEC_{50} < 0.3$, which corresponds to the range of >0.5 -fold and <2 -fold change in EC_{50} compared to the WT receptor. Mutations that increase the receptor sensitivity toward remdesivir ($\Delta pEC_{50} > 0.3$) but not toward the UT2 ($\Delta pEC_{50} < 0$) are marked in red. Mutations that increase the receptor sensitivity toward both remdesivir and UT2 ($\Delta pEC_{50} > 0.3$ for remdesivir, $0 < \Delta pEC_{50} < 0.3$ for UT2) are marked in green. All experiments were performed in triplicate, and the data are expressed as the means. **(b)** Allele frequencies of the four gain-of-function mutations (G68^{1.49}C, D130^{3.32}G, V159^{34.54}M, and A249^{ICL3}G). Data are modified from the 14KJPN Genome Reference Panel (<https://jmorp.megabank.tohoku.ac.jp/202112/>).

Supplementary Figure 5. Original Western Blots

The original blots for Figures 1d; 3a; 4c; 4d; Supplementary Figures 1d; 1e; 3a; 3b; 3g.

